REMARKS

Applicants have made a significant contribution to the art of new gene discovery by overcoming many of the shortcomings of earlier methods such as repeated sequencing of known or previously-identified gene sequences and loss of low abundancy clones

There are a number of advantages of the present invention compared to prior art methods. For example, methods using selective hybridization in solution, as used in cDNA normalization or subtraction procedures (e.g., as described by Kayne et al.), often result in the loss of certain low abundant clones. In contrast, using the iterative microarray-based subtraction approach, all clones in the library are efficiently identified. For example, a low abundant cDNA clone is found after multiple iterative rounds of hybridization with a progressively larger pool of redundant and previously-identified sequences. Another advantage of the claimed methods is that the resulting cDNA clones are more likely to be near full length. In contrast, other methods for subtraction and normalization often enrich short, incomplete cDNAs (page 11, lines 1-9, of the specification).

The claims have been amended to distinguish the invention over prior art methods. Upon entry of this amendment, claims 58-62 are pending. The new claims are supported by disclosure at page 4, lines 3-24; page 7, lines 1-17 and line 26; page 8, lines 16-28; page 11, lines 10-30; and page 20, lines 7-20, of the specification.

No new matter has been added.

The Examiner has indicated that the figures are objected under 37 CFR § 1.84. In response, Applicants file herewith replacement Figures 1-4 in compliance with 37 CFR § 1.84. This objection can be withdrawn.

35 USC § 102 (b)

Claims 48 and 52-57 have been rejected under 35 USC § 102 (b) as anticipated by Heller et al. (PNAS USA 94: 2150-5 (1997)).

Heller et al. describe a microarray of known human genes, which are suspected of playing a role in rheumatoid arthritis (RA). Fore example, IL-2, GM-CSF, as well as control cDNAs such as housekeeping genes actin and GAPDH were immobilized on a solid support and allowed to hybridize with diseased tissue-derived nucleic acids. Differential and quantitiative expression of each of the known genes thought to be implicated in diseased RA tissue as well as inflammatory bowel disease was measured by detecting the intensity of the hybridization signal. Levels of expression of implicated genes (e.g., cytokines, chemokines, growth factors) were normalized by comparison to the level of hybridization to immobilized housekeeping genes such as actin and GAPDH.

The new claims are drawn to a method of enhancing the rate of discovery of novel gene sequences by reducing or eliminating sequencing of redundant genes such as housekeeping genes. The method required by the new claims differs significantly from the method of Heller et al. Heller et al. describes microarray immobilization of known genes such as cytokines, chemokines, growth factors, and housekeeping genes, whereas the claims require immobilization of a population of previously-uncharacterized genes. Moreover, the claims require a subtraction pool of "redundant genes" in solution and a progressive enlargement of the pool of known or "redundnant" genes, whereas Heller et al. simply use redundant (housekeeping genes) as a control immobilized on the microarray to normalize differential gene expression.

In view of these fundamental differences between the claimed method and Heller's

methods, Applicants submit that the new claims are novel over Heller et al.

35 USC §103 (a)

The Examiner stated two grounds of rejection under USC §103. First, the claims were rejected for obviousness over Heller et al.; and second, the rejection for obviousness over Kayne et al. in view of Gress et al. was maintained. The rejected claims were canceled. The prior art references are addressed below with respect to the new claims.

Heller et al.

Claims 49-51 were rejected under 35 USC § 103 (a) as obvious over Heller et al.

Heller et al. fail to describe or suggest the methods as now claimed. The rejected claims were canceled. There is no suggestion in Heller et al. to identify novel genes, much less enhance the rate of their discovery by reducing the level of redundancy of known sequences in a population of previously-uncharacterized nucleic acids. Heller et al. also fail to describe or suggest probing arrays derived from the same uncharacterized population with progressively larger and larger numbers of redundant or known sequences. The new claims are therefore nonobvious in view of Heller et al.

Kayne et al. in view of Gress et al.

Claims 27-33, 41-47 and 52-54 were also rejected for obviousness over Kayne et al. in view of Gress et al. Applicants have cancelled claims 27-33, 41-47 and 52-54.

The new claims are drawn to an iterative method of eliminating the frequency of redundant sequences (e.g., housekeeping genes) to reduce the number of nucleic acids to be sequenced in efforts to identify novel gene sequences. According to the claimed methods, a population of previously-uncharacterized nucleic acids are immobilized and allowed to hybridize

with a solution of known or redundant genes such as a housekeeping genes. The redundant nucleic acids in the "initial subtraction pool" hybridize to complementarey nucleic acids, which are immobilized on an array. By utilizing an ordered array of DNA samples, the position of each sample is tracked and linked to the original sample from which the DNA on the array was generated. The positions with a high intensity signal represent a known gene in the "subtraction probe pool" – these positions therefore do not merit sequencing, because their sequence has already been identified. Some fraction of immobilized nucleic acids on the array don't hybridize at all or hybridize weakly – these nucleic sequences merit sequencing, because they are more likely to represent novel genes. Once sequenced, these "newly-identified" sequences are added to the first "subtraction probe pool", because (following sequencing) they now represent a known or redundant gene, i.e., there is no reason to sequence DNA from this clone again. The process of adding newly-identified gene sequences to the "subtraction probe" is repeated, so that the pool of known or redundant sequences, becomes larger and larger. The positions, which correspond to non-hybridizers or weak hybridizers, i.e., nucleic acids to be sequenced, becomes smaller and smaller until all the sequence of all the nucleic acids in the "previously-uncharacterized population" are identified. Such a population is known as a Unigene set.

As described in the paragraph spanning pages 11-12 of the specification, by adding the newly identified nucleic acid fragments to a previous subtractive probe pool, subsequent microarrays of randomly-chosen clones from a nucleic acid population are exposed to a larger number of labeled probes (representing redundant/known genes). Accordingly, the method of the amended claims eliminates the re-sequencing of known clones – a problem that reduces the efficiency of prior art methods. As the number of labeled probed increases with successive

iterations, the number of labeled "known" clones increases and fewer clones will be unlabeled. Eventually, with sufficient iterations, all of the clones in microarrays of randomly-chosen clones from a given library will be labeled, as all of the clones will have been sequenced.

As discussed above, the claimed methods include a previously undescribed series of steps, which lead to enhancement of the rate of new gene discovery by reducing the number of candidate nucleic acids to be sequenced.

With respect to the combination of Kayne and Gress, the Examiner states:

> It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Kayne et al. so as to have spotted the library of random nucleic acids on the microarray in order to have provided an improved method for isolating and identifying non-redundant nucleic acids. (paragraph spanning pages 14-15 of Paper No. 22).

Gress et al. fail to described the elements of the amended claims, which are missing in the Kayne et al. reference. Gress et al. describe a method of determining a pattern of transcription to identify "sequences abundantly expressed in several tissues". This was done by immobilizing 2 cDNA libraries (uncharacterized sequences) onto a filter and probing the blots with a labeled cDNA pool made from RNA extracted from a set of mouse tissues (unknown sequences) (page 610, Materials and Methods, of Gress et al.). Analysis of total cDNA pools derived from different tissues was used to identify clones on cDNA library arrays containing mRNA sequences expressed at middle to high abundance.

Gress et al. fail to describe or suggest probing the immobilized library with known/redundant sequences. These researchers also fail to describe or suggest a critical element of the claimed invention – progressively increasing the size of the hybridization probe pool as more and more sequences become identified.

None of the prior art references alone describes all of the required steps of the amended claims, and the combination fails to suggest certain critical steps of the invention.

Indefiniteness

Claims 27-57 are rejected under 35 USC § 112, second paragraph as being indefinite. In view of the cancelation of claims 25-57, this rejection is moot.

Written Description.

Claims 27-57 were rejected under 35 USC § 112, first paragraph for lack of written description. The rejected claims were canceled.

Enablement.

Claims 27-57 are rejected under 35 USC § 112, first paragraph for lack of enablement in regard to the rectitation of the phrases "on said array" and "without isolating said sequence." The claims no longer recite these phrases; therefore, this rejection should be withdrawn.

CONCLUSION

Applicants submit that the application is in condition for allowance and such action is respectfully requested.

Applicants submit herewith a Petition for a Three-Month Extension of Time, along with the appropriate fee. With this extension of time, these documents are due on or before February 27, 2003. The Commissioner is hereby authorized to charge any additional fees that may be due, or credit any overpayment of same, to Deposit Account No. 50-0311, Reference No. 21127-501 RCE.

Should any questions or issues arise concerning the application, the Examiner is encouraged to contact Applicant's undersigned attorney at the telephone number indicated below.

Respectfully submitted,

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EXHIBIT A

Marked up Version

Cancel claims 27-57. Add new claims 58-62.

--58. (new) A method of enhancing the rate of novel gene discovery in a population of previously-uncharacterized nucleic acid molecules, comprising

a. contacting an first ordered array of nucleic acid samples from said population with an initial complex subtraction probe pool, said probe pool comprising a labeled probe corresponding to a redundant or known sequence;

b. identifying a nucleic acid, said nucleic acid being characterized by weak or no hybridization to said initial probe pool;

c. sequencing said nucleic acid to yield a newly-identified sequence;

d. adding said newly-identified sequence to said initial pool to generate a second complex subtraction probe pool, said second pool comprising a larger number of labeled probes compared to said initial pool;

e. contacting a second ordered a array of nucleic acid samples from said population with said second subtraction probe pool; and

f. repeating steps b.-e., wherein each repeat of steps b.-e. reduces the redundancy of said population of nucleic acid molecules, thereby enhancing the rate of novel gene discovery.--

--59. (new) The method of claim 58, wherein said redundant or known sequence is selected from the group consisting of a public database member gene, a housekeeping gene or ribosomal gene.--

--60. (new) The method of claim 58, wherein said first and said second ordered array comprise DNA immobilized on a glass surface.--

--61. (new) The method of claim 58, comprising repeating steps b.-e. to generate Unigene set.--

--62. (new) The method of claim 58, wherein said weak hybridization comprises a signal-to-noise ration of less than 0.5.--